

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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|----------------------------------------------------|---|--------------------------|
| In re Application of: <i>Patrice Marche et al.</i> |) | Confirmation No. 7396 |
| |) | |
| Application No. 10/581,814 |) | Art Unit: 1637 |
| |) | |
| Filed: August 22, 2007 |) | Examiner: S. C. Woolwine |
| |) | |
| For: Method for quantitative evaluation of a |) | |
| rearrangement or a targeted genetic |) | |
| recombination of an individual and uses |) | |
| thereof |) | |

DECLARATION UNDER 37 C.F.R. § 1.132

I, the undersigned, Nicolas PASQUAL, do hereby declare that:

1. I am a citizen of France, residing at 43 allée des romantiques 38100 Grenoble.
2. I am currently employed at Immun'ID. My curriculum vitae is enclosed.
3. I have read the specification of U.S. Patent Application 10/581,814. I have reviewed the Office Action, dated July 13, 2010. I have also read the cited references used to allege that the claimed invention is obvious, as set forth in the Office Action dated July 13, 2010. I believe that the feature of determining a TCRAD rearrangement from a sample of human genomic DNA by multiplex PCR is not obvious over these references.
4. The Office Action of July 13, 2010, alleges that the methods of Pasqual can be obviously adapted to perform multiplex PCR on a sample of up to 35 kilobases as disclosed by Barnes at 68 °C for 11-24 minutes and expect to directly visualize an obtained product in a gel stained with ethidium bromide. I believe and I have tested as to whether a high concentration of genomic DNA (gDNA) as present in the pooled samples described by Pasqual can be efficiently run through a multiplex PCR protocol for a PCR fragment over 35 kilobases as described by Barnes. Based on my results described below, I have determined that such methods cannot obviously be combined to effectively produce a PCR product.
5. For experimental conditions, gDNA obtained from fish as described in the table below was placed in a test tube along with primers to amplify a product of p53 using primers specific for the p53

gene. The size of the intended PCR products based on these primers is 400 bases in length. To each tube a polymerase and magnesium chloride was also added. To control for the viscosity of DNA in the sample, each tube received 25 ng of murine gDNA. Each tube had an equal final volume of 20 μ L.

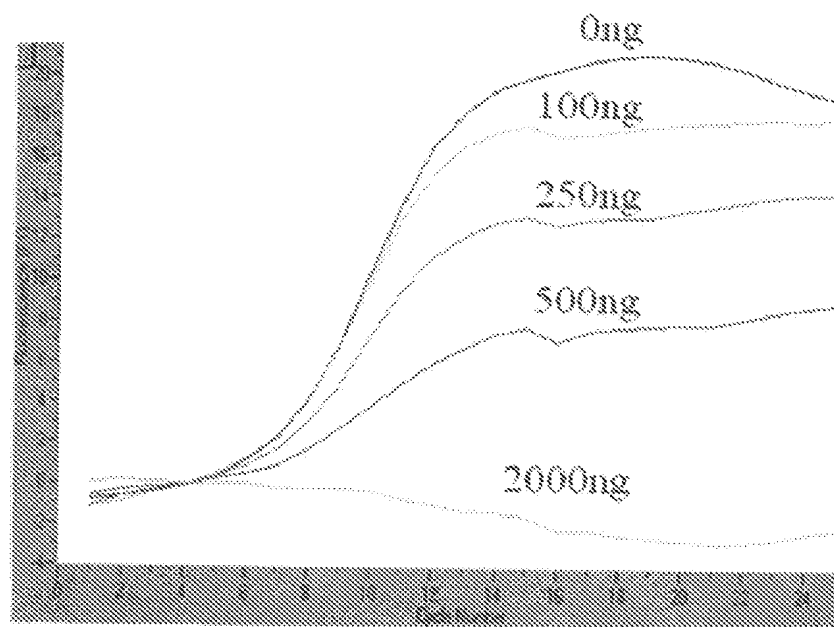
TABLE 1

| | | | | | |
|----------------------------------------------|-----------------|-----------------|-----------------|-----------------|------|
| Concentration of the source of fish gDNA | 400 ng/ μ L | 100 ng/ μ L | 100 ng/ μ L | 100 ng/ μ L | 0 |
| Volume of fish gDNA | 5 | 5 | 2,5 | 1 | 0 |
| Final quantity of fish gDNA in each reaction | 2000 ng | 500 ng | 250 ng | 100 ng | 0 ng |
| QSP H ₂ O (μ L) | 0 | 0 | 2,5 | 4 | 5 |

6. For the PCR cycling, initial degradation was performed for 3 minutes at 95 °C, and then for thirty cycles, each cycle being 7 seconds at 60 °C and 8 seconds at 72 °C followed by measurement of fluorescence.

7. The results from this experiment, depicted in the graph below, clearly demonstrate that as the concentration of gDNA present in a sample increases, the efficiency of the PCR decreases.

FIGURE 1



8. Accordingly, as the concentration of gDNA in the sample increases, so too does the steric hinderance on the ability of the polymerase to efficiently produce a fragment. When the total amount of DNA in the tube is 2 μ g, the PCR is totally inhibited.

9. I further declare that to obtain a copy number, for a rearranged gene, equivalent to the copy number of phage genome present in the experiments performed by Barnes, far more than 2 μ g of human genomic DNA would be required. In view of these data, one skilled in the art would not be motivated to attempt to perform methods for producing a PCR fragment as long as 35 kilobases (as disclosed by Barnes), with human gDNA as starting template. Accordingly, at the time of filing of the present application, the claimed invention was not obvious.

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 06/01/2011
JAW/STY

By: Nicolas PASQUAL

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Nicolas Pasqual, PhD, has served as ImmunID Technologies CSO since its creation, then President & CEO. He has a strategy leadership in the company's scientific and economic development. Inventor of the company's proprietary technology, Dr. Pasqual orchestrates industrial and academic partnerships.

He has 9 years of immunomonitoring skills working in France (CEA, Grenoble, France), Canada (Hôpital Ste Justine Montréal, Canada) and management (High Management School EM Lyon, France). He started in fundamental research (immuno-genetics, virology & microbiology) and was in charge of technology transfer projects. He is the author of several scientific publications as well as four patents in the field of immunological analysis and pre-analytical process. He participates in the organization of events aimed at strengthening the relationship between biotechnology companies and the "Société Française d'Immunologie" (SFI). Dr. Pasqual is currently involved in immunomonitoring axes at the LyonBiopôle cluster and at Canceropole CLARA, as well as a member of the LyonBiopôle economic development group and of the bioentrepreneur club of ADEBAG/Grenoble.

As a bioindustry thought actor, Dr. Pasqual speaks frequently on innovation and entrepreneurship strategies. His main motivation is to help patients and physicians day to day work, by improving medicine tools thanks to immunomonitoring democratization's. According to him, innovative immunomonitoring must be as "easy & ready to use" as possible. For this, NP is actively implicated in partnerships program with pharmaceutical groups like Sanofi-Pasteur, Transgen, Innate Pharma, LFB, Cytheris, Charles River... and academic center like the CEA, INSERM, Marie-Lannelongue hospital (Paris), Léon Bérard Center (Lyon), La pitié Salpêtrière (Paris), and Hospices civils de Lyon (HCL).



Research experience

2004-2010 President, CEO & CSO of ImmunID Technologies www.immunid.com
GRENOBLE & LYON France

Immunomonitoring of the specific immune system. Development of innovative diagnostic solution.

General company activity

ImmunID offers innovative diagnostic and prognostic tools and high value-added services

-to contribute to the selection and development of the best molecules to help pharmaceutical companies measure their efficiency.

-to define immune signatures and monitor immunoregulation status and contribute to delineating stages of disease progression and to choosing, adjusting and following appropriate therapies.

These programs prove their usefulness in personalized medicine for patient follow-up and classification in diverse clinical fields including immune-related diseases, such as infectious diseases, blood cancers or solid cancers, graft versus host reaction, and graft rejection.

Applications

ImmunID strives to develop gold standard tools for monitoring the immune cell repertoire. Thanks to genome sequencing and immunogenetics, it is possible to study the genes that form the basis of immune repertoire diversity. ImmunID has developed technologies to produce pictures of the T and B cell receptor repertoires, and to diagnose subtle changes in the immune repertoire at an early stage. These "immune signatures" can then be used as an indicator to follow vaccine or drug efficiency or to monitor the health status of a patient.

2000/2003 Ph.D. : *From the experimental TCR α chain rearrangement analyse to the description of TCRAD locus regulation*

Laboratory of Molecular and Cellular Immunology, C.E.A.-Grenoble, FRANCE

1999/2000 D.E.A. : *Study of TCR gene rearrangement and thymus development*

Laboratory of Molecular and Cellular Immunology, C.E.A.-Grenoble, FRANCE

1998/1999 Master of Science, *IL-15 effect on NK anti-tumoral response*

Hospital St Justine Montreal, QC, CANADA

Skills

| | |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domains | Management, Strategy, Finance, VCs funding. Immunology, immunogenetic, Molecular Biology, Cellular Biology, Biomarkers, Diagnostic, personalized Medicine, |
| Techniques | MOLECULAR : Multiplex, QPCR and RT-PCR CELLULAR : cytometry, cells sorting, cell culture, FTOC |
| Languages | French Native Fluent English. Skills Italian. |

Publications

Optimal epitope composition after antigen screening using a live bacterial delivery vector
Application to TRP-2

Madiha Derouazi, Yan Wang, Raphaël Marlu, Olivier Epaulard, 1Jean-François Mayol, 2Nicolas Pasqual, Audrey Le Gouvellec, Benoit Polackland Bertrand Toussaint1 Bioengineered Bugs
March 2010 1:1, 1-10

Numerical modelling of the V-J combinations of the T cell receptor TRA/TRD locus.

Thuderoz F, Simonet MA, Hansen O, Pasqual N, Dariz A, Baum TP, Hierle V, Demongeot J, Marche PN, Jouvin-Marche E. PLoS Comput Biol. 2010 Feb 19;6(2):e1000682.

High diversity of the immune repertoire in humanized NOD.SCID,gamma c-/- mice.

Marodon G, Desjardins D, Mercey L, Baillou C, Parent P, Manuel M, Caux C, Beillier B, Pasqual N, Klatzmann D. Eur J Immunol. 2009 Aug;39(8):2136-45.

Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome.

Gobert M, Treilleux I, Bendriss-Vermare N, Bachelet T, Goddard-Leon S, Arfi V, Biota C, Doffin AC, Durand I, Olive D, Perez S, Pasqual N, Faure C, Ray-Coquard I, Puisieux A, Caux C, Blay JY, Ménétrier-Caux C. Cancer Res. 2009 Mar 1;69(5):2000-9. Epub 2009 Feb 24.

Analysis of the TCR alpha-chain rearrangement profile in human T lymphocytes.

Fuschiotti P, Pasqual N, Hierle V, Borel E, London J, Marche PN, Jouvin-Marche E. Mol Immunol. 2007 Jul;44(13):3380-8. Epub 2007 Mar 27.

IMGT/GenetInfo: T cell receptor gamma TRG and delta TRD genes in database give access to all TR potential V(D)J recombinations.

Baum TP, Hierle V, Pasqual N, Bellahcene F, Chaume D, Lefranc MP, Jouvin-Marche E, Marche PN, Demongeot J. BMC Bioinformatics. 2006 Apr 26;7:224.

Role of the T cell receptor alpha chain in the development and phenotype of naturally arising CD4+CD25+ T cells

Bosco N, Hung HC, Pasqual N, Jouvin-Marche E, Marche PN, Gascoigne NR, Ceredig R. Mol Immunol. 2006 Feb;43(3):246-54.

Quantitative analysis of ADV families utilization in mouse CD4+ and CD8+ T cell subpopulations in the thymus and periphery.

N. Pasqual, P. Fuschiotti, S. Cadau, E. Borel, F. Thuderoz ‡, J. Demongeot ‡, P. N. Marche & E. Jouvin-Marche Immunology 2004

IMGT/GenetInfo: enhancing V(D)J recombination database accessibility

Baum TP, Pasqual N, Thuderoz T, Hierle V, Chaume D, Lefranc MP, Jouvin-Marche E, Marche PN, Demongeot J Nucleic Acids research 2004 janv vol 32 D51-54

Quantitative and qualitative changes in V-J alpha rearrangements during mouse thymocytes differentiation: implication for a limited T cell receptor alpha chain repertoire.

Pasqual N, Gallagher M, Aude-Garcia C, Lolodice M, Thuderoz F, Demongeot J, Ceredig R, Marche PN, Jouvin-Marche E. J Exp Med 2002 Nov 4;195(9):1163-73

Bcl-2/Bax protein expression in heart, slow-twitch and fast-twitch muscles in young rats growing under chronic hypoxia conditions.

Riva C, Chevrier C, Pasqual N, Saks V, Rossi A. Mol Cell Biochem. 2001 Oct;226(1-2):9-16.